Immunologic and Clinical Effects of Targeting PD-1 in Lung Cancer

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Therapeutic antibodies that block the programmed cell death protein-1 (PD-1) immune checkpoint pathway prevent T-cell downregulation and promote immune responses against cancer. Several PD-1 pathway inhibitors have shown robust activity in initial trials. This article reviews the preclinical evidence, rationale, and clinical pharmacology of blockade of PD-1 or its ligands as therapy for lung cancer and provides an overview of agents in development, clinical evidence to date, and implications for clinical application.

BACKGROUND

First-line therapy for advanced non–small cell lung cancer (NSCLC), which accounts for ~85% of all lung cancers, is platinum-based chemotherapy.[1,2] Patients with specific mutations may effectively be treated with targeted agents initially.[2,3] However, most develop resistance to these therapies, with subsequent disease progression.[2,3] As such, the average 5-year survival rate is 4% for patients diagnosed with advanced disease, highlighting a great need for improved treatment options.[1] Immunotherapy is effective in patient subsets in some cancers (e.g., melanoma and renal cell carcinoma) and can increase survival.[4–6] However, the limited activity of bacille Calmette-Guérin vaccination, interleukin (IL)-2, and interferons in clinical trials has promoted the perception that NSCLC is not an immunoresponsive tumor.[7] Different immunologic approaches targeting immune checkpoint pathways are showing promise in development, and preclinical and clinical evidence provides rationale for investigating these newer immunotherapies in NSCLC and other tumors.

RATIONALE FOR IMMUNE CHECKPOINT INHIBITION

Upon emerging from the thymus, naive T cells circulate in blood through lymph nodes and seek foreign ("nonself") antigens presented by specific antigen-presenting cells, typically dendritic cells.[8] T cells can recognize not only pathogen-associated antigens but also abnormally expressed self-proteins—indicating mutated or transformed tumorigenic cells—as "nonself." If T cells encounter their specific antigen in the context of appropriate costimulatory molecules, the cells become activated and upregulate activation and homing molecules. These T cells, termed effector T cells, are able to enter inflamed tissues in search of infected or cancerous cells. Among other functions, effector T cells can produce inflammatory cytokines and/or cytolytic granules, leading to apoptosis or necrosis of infected or tumor cells. Throughout the duration of an immune response, local and systemic downregulatory forces are in play to minimize damage to healthy cells and tissues. These can involve immunosuppressive cytokines, regulatory T cells (Tregs), and negative signaling from other cells.

Immune checkpoint pathways

Immune checkpoint pathways strongly downregulate T-cell activation with the intent of keeping nascent T-cell responses in check and reducing the likelihood of an immune attack against normal tissues. During tumorigenesis, however, cancer cells may exploit these co-inhibitory pathways to resist detection or avoid elimination by the adaptive immune system.[8,9] The programmed cell death protein-1 (PD-1) is a critical checkpoint molecule that is expressed by T cells upon activation. The PD-1 checkpoint pathway is thought to act primarily in peripheral tissues to dampen ongoing immune responses and/or to prevent damage to self-tissues.[9] PD-1 is expressed by B cells, natural killer (NK) cells, dendritic cells, and activated monocytes, in addition to T cells. PD-1 ligands—which include PD-L1 and PD-L2, among others—are expressed by macrophages and monocytes, and these can be induced in numerous cell types in an inflammatory environment.[10]

The ability of nonimmune cells to express ligands for PD-1, primarily PD-L1, is exploited by tumors as one way to avoid immune attack.[11,12] Tumor cells can also downregulate antigen

expression to avoid detection. In addition, production of immunosuppressive mediators and retention of Tregs and immune suppressor cells within the tumor microenvironment can dampen antitumor immune responses.[11]

This article focuses on the PD-1 pathway as a novel therapeutic target for oncology drug development.

RATIONALE FOR PD-1 ANTAGONISM
PD-1 pathway and its role in cancer
Although most understanding of basic and tumor immunology comes from academic research, evidence from the clinic supports a role for the PD-1 pathway in human cancers. PD-L1 expression has been detected in lung, ovary, renal, and colon carcinomas and in malignant melanoma but not in normal tissues, including the lung, uterus, kidney, colon, or skin (nevi).[13–15] PD-L1 expression by tumor cells is associated with a worse prognosis in breast cancer, gastric cancer, esophageal cancer, hepatocellular carcinoma, malignant melanoma, ovarian cancer, pancreatic cancer, renal cell carcinoma, and urothelial cancer.[12]

There is also evidence that human tumors can express PD-L2.[16,17] NSCLC-associated fibroblasts constitutively express both PD-L1 and PD-L2. Decreased survival in patients with PD-L2–positive (vs. PD-L2–negative), esophageal, ovarian, or hepatocellular cancer has also been described. PD-1:PD-L2 binding has higher affinity and is slightly different than PD-1:PD-L1 binding, although whether this translates to different T-cell signaling and antitumor effects is unclear.[16]

If PD-1 ligands are involved in downregulating antitumor immune responses, then they would likely be acting on tumor-specific PD-1–expressing T cells. In support of this hypothesis, in both NSCLC and melanoma patients, higher levels of PD-1 were observed on tumor-infiltrating lymphocytes (TILs) than on circulating lymphocytes.[14,18] Furthermore, in the peripheral blood of vaccinated melanoma patients, both melanoma antigen–specific cytotoxic lymphocytes and Tregs expressed PD-1.[19] Finally, there was a negative correlation between tumor PD-L2 expression and the presence of CD8+ TILs in esophageal cancer.[16]

Preclinical support for PD-1/PD-L1 antagonism as a therapeutic intervention
Animal studies have suggested that the PD-1 pathway is involved in tumor immune evasion and that blockade of the PD-1 pathway can restore antitumor immune responses. Tumor cells expressing PD-L1 had increased resistance to T cell–mediated lysis and showed enhanced tumorigenesis and invasiveness, as compared with tumor cells lacking PD-L1 expression. These effects were reversed by administration of anti-PD-L1 antibody.[20] Separately, growth of tumor cells was inhibited in PD-1-deficient mice, suggesting a strong antitumor immune response in the absence of PD-1:PD-L1 interactions.[20,21] Furthermore, in mice with established tumors, administration of anti-PD-1 and/or anti-PD-L1 antibodies led to reduced tumor burden and increased survival.[22,23]

PD-1 pathway blockade may enhance other immunotherapeutic approaches, including those using nonoverlapping immune checkpoint pathways. As evidence, increased antitumor activity—relative to either single agent—was seen when anti–cytotoxic T lymphocyte–associated antigen 4 and anti-PD-1 antibodies were used concurrently, whereas sequential use showed efficacy similar to that of the single agents.[24,25] Similarly, combination treatment with both anti–lymphocyte activation gene-3 (another T-cell-inhibitory receptor) and anti-PD-1 antibodies cured most mice of established tumors that were largely resistant to single-antibody treatment.[26] In addition, there is preclinical evidence to suggest that PD-1 pathway blockade could be combined with other immunotherapeutic approaches, including administration of ILs, innate immune modulators, or vaccines. Combination of anti-PD-1 with recombinant IL-21 led to enhanced antitumor activity, with strong tumor growth inhibition and complete regression in the majority of mice.[27] In a model of chronic viral infection leading to T-cell exhaustion, similar to tumor-mediated immunosuppression, combination treatment with anti-PD-1 and IL-2 was synergistic. It increased both the number of virus-specific CD8+ T cells and the ability of CD8+ T cells to kill infected cells.[28] In other tumor models, use of anti-PD-L1 antibody with transfer of tumor-specific T cells led to eradication of PD-L1-expressing squamous cell carcinomas, in contrast to no tumor eradication with T-cell transfer alone.[29] Combined treatment of anti-PD-1 antibody with CpG (a Toll-like receptor agonist) or with granulocyte macrophage colony-stimulating factor–secreting tumor cell immunotherapy prolonged the survival of tumor-bearing animals.[30,31] Finally, multiple vaccination approaches had augmented responses when PD-1–pathway blockade was used.[25,32–34]

Studies of human antitumor responses provide some insight regarding why the PD-1 pathway seems to be a promising target for immunotherapy in solid tumors, including lung cancers. CD8+ TILs isolated from NSCLCs had increased expression of PD-1 and impaired functional responses (in vitro proliferation and inflammatory cytokine production) as compared with circulating CD8+ T cells or CD8+ T cells from healthy volunteers. Addition of anti-PD-L1 antibody significantly improved the ability of the CD8+ TILs to proliferate and produce interferon-γ in vitro.[18] In a similar study using cultures of tumor-derived dendritic cells and TILs from ovarian cancer patients, addition of anti-PD-L1 antibody significantly increased interferon-γ production by TILs in response to tumor antigens. When these TILs were transferred to immunodeficient mice bearing the ovarian tumors, reduced tumor growth was seen as compared with that of mice in control groups.[35]

Some of the studies in which anti-PD-1 antibody enhanced antitumor vaccination responses also demonstrated immunologic changes that might help explain the apparent additive effects. In a short-term in vitro assay used to generate melanoma-specific T cells, addition of an anti-PD-1 antibody promoted the generation of melanoma antigen–specific cytotoxic lymphocytes and reduced their inhibition by Tregs.[19] T cells isolated from melanoma antigen–vaccinated patients and exposed to melanoma antigens in vitro had augmented activation and expansion of functional effector cytotoxic lymphocytes when cultured in the presence of an anti-PD-1 antibody.[36]

In an *in vitro* immunization model using fused dendritic cells and tumor cells, anti-PD-1 antibody promoted T-cell polarization toward an activated phenotype and increased killing of tumor target cells.[37]

The PD-1 pathway may also be used by the innate immune system in some cancers. NK cells from multiple myeloma patients expressed PD-1, whereas NK cells from healthy patients did not.[13] Treatment of PD-1+ NK cells with an anti-PD-1 antibody improved *in vitro* antitumor effects, including enhanced interferon-γ production and cytotoxic functions.[13]

Immunologic studies have identified potential mechanisms for these clinical responses. In the above-described preclinical studies, blockade of the PD-1 pathway led to the following:

- Increased numbers of effector T cells through induction or expansion[14,18,19,21,30–32,35]
- Augmented cytolytic activity of tumor-specific cells, leading to improved tumor lysis[14,22,30,36–38]
- Enhanced production of proinflammatory cytokines[14,18,19,21,23,30,34,35,38]
- Accumulation of effector T cells in the tumor sites via increased homing or persistence[21,30,32,38]
- Reduced numbers of Tregs at the tumor site or reduced activity of Tregs[19,31,32,37]
- Downregulation of potentially suppressive cytokines (IL-10)[23]

Figure 1 depicts the role of the PD-1 pathway in tumor immune evasion and the mechanism of action of PD-1 pathway blockade.[39]

PD-1 PATHWAY INHIBITORS IN CLINICAL DEVELOPMENT

A number of promising agents targeting the PD-1 pathway are in clinical development: AMP-224, BMS-936559, MEDI4736, nivolumab, pembrolizumab (MK-3475), and pidilizumab (CT-011) (**Table 1**). Clinical trial data of these PD-1 pathway inhibitors in lung cancer, to date, are shown in **Tables 2** and **3**. AMP-224 and pidilizumab do not currently have any trials in lung cancer, and MEDI4736 currently has an ongoing phase I trial in multiple advanced cancers, including NSCLC.[40] For all immune checkpoint inhibitors investigated to date, the safety profiles were largely characterized by immune-related adverse events (irAEs), in keeping with their mechanisms of action (**Tables 2** and **3**). Tolerability of the immune checkpoint inhibitors was generally good, with few dose-limiting toxicities reported.[41–49] The irAEs are discussed in more detail below. Preliminary data suggest that PD-1 pathway inhibitors have activity in lung cancer.

PD-L1

Agents that target PD-L1 will inhibit PD-1:PD-L1 binding, as well as PD-L1 binding to CD80 on T cells.[9] In the phase I trial of the PD-L1 inhibitor BMS-936559, objective responses were seen in 10% of patients (5 of 49) with advanced NSCLC (**Table 2**).[42] When results were broken down by histological subtype, 1 of 13 evaluable patients with squamous NSCLC had an objective response, 3 patients had stable disease lasting ≥24 weeks, and

6 patients had progression-free survival at 24 weeks. Of 36 evaluable patients with nonsquamous subtype, 4 had an objective response, 3 had stable disease lasting ≥24 weeks, and 9 patients had progression-free survival at 24 weeks.

MPDL3280A, another antibody targeting PD-L1, showed an objective response rate of 23% (12/53) in patients with NSCLC who had been previously treated, with 55% having received at least 3 prior regimens (NCT01375842) (**Table 2**).[45] In the interim analysis, clinical responses were seen in patients with both squamous and nonsquamous histologies, regardless of epidermal growth factor receptor mutation status. At the time of analysis, responses were ongoing in all but one responder with lung cancer; the duration of responses ranged from >24 to >75 weeks. On the basis of these promising results, two phase II trials are under way in NSCLC. The first involves monitoring objective responses of patients with PD-L1+ locally advanced or metastatic NSCLC receiving MPDL3280A monotherapy (NCT01846416). The second involves assessing overall survival vs. docetaxel in patients with advanced or metastatic NSCLC who failed platinum therapy (NCT01903993).[40]

PD-1

Antibodies targeting PD-1 will inhibit binding of PD-1 to both its ligands, PD-L1 and PD-L2. A humanized immunoglobulin G (IgG)-4 anti-PD-1 antibody, pembrolizumab (MK-3475), is being evaluated in an ongoing phase I trial in patients with advanced solid tumors, including patients with NSCLC (NCT01295827) (**Table 3**).[43] In an interim analysis of previously treated NSCLC patients, the objective response rate was 21% overall using the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1, and most responses were observed by 9 weeks. At the time of data cut, the preliminary median durations of overall survival and progression-free survival were 51 and 9.7 weeks, respectively.

A phase I trial enrolling patients with advanced NSCLC evaluating pembrolizumab in combination with cisplatin/pemetrexed or carboplatin/paclitaxel (NCT01840579) is ongoing. In addition, overall survival, progression-free survival, and safety of pembrolizumab (low dose or high dose) vs. docetaxel are being evaluated in a phase II/III trial in previously treated NSCLC patients (NCT01905657). Two more studies are planned that will start enrolling patients with advanced NSCLC later this year. A phase I/II study will evaluate the safety, tolerability, and efficacy of pembrolizumab in different combinations with chemotherapy, targeted agents (bevacizumab, erlotinib, and gefitinib), and ipilimumab (NCT02039674); and a phase I study will study responses and safety of pembrolizumab monotherapy in patients with PD-L1+ tumors (NCT02007070).[40]

Nivolumab, a fully human IgG4 PD-1 immune checkpoint inhibitor antibody, has undergone the most extensive clinical evaluation in lung cancer among the PD-1 pathway inhibitors. Evidence of activity both as a monotherapy in squamous and nonsquamous NSCLC and in combination with conventional chemotherapy has been demonstrated in patients with NSCLC (**Table 3**).[50,51] In pretreated advanced NSCLC patients, nivolumab monotherapy had an overall response rate of
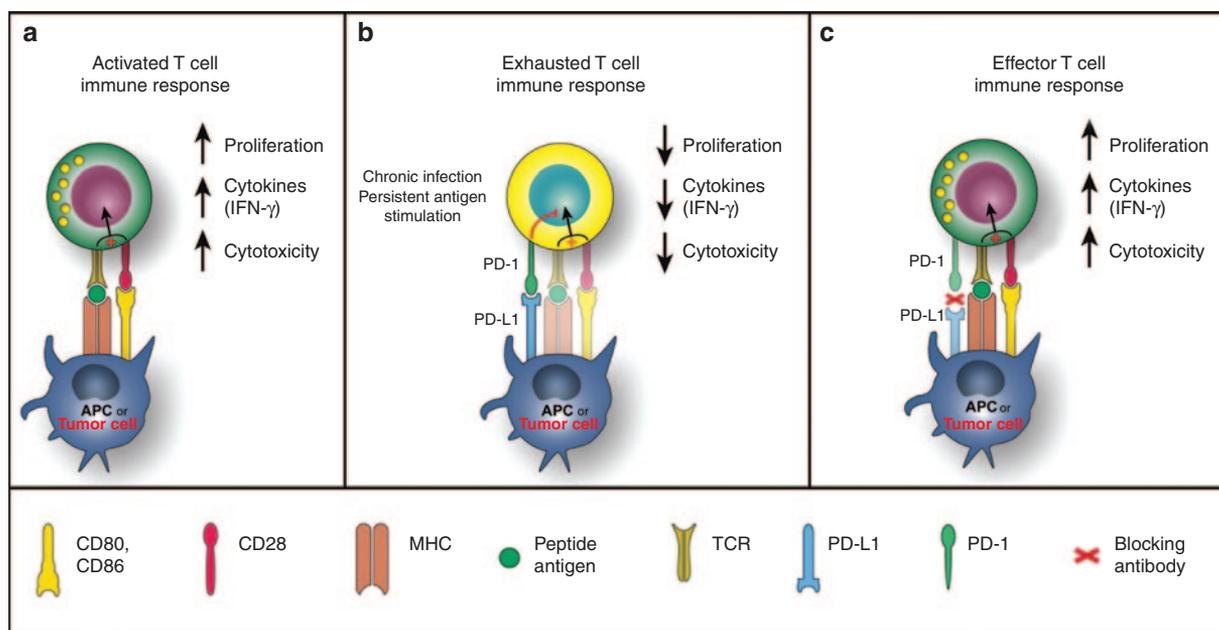
Figure 1 PD-1 in T-cell activation, exhaustion, and effector function. (**a**) T cells are activated via (i) binding of MHC plus peptide on an APC to the TCR and then (ii) binding of APC CD80/86 to T-cell CD28. In patients with cancer, tumor cells can also serve as APCs. Upon T-cell activation, PD-1 expression is induced. (**b**) In situations of chronic infection or persistent stimulation, PD-L1 signals through T-cell PD-1 to "turn off" T cells in order to minimize damage to healthy tissue (activation signaling is blocked). Tumor cells can upregulate PD-L1 in order to "turn off" T cells that might destroy them. (**c**) Blocking the PD-1/PD-L1 signaling pathway allows T cells to maintain their effector functions. In patients with cancer, activated tumor-specific T cells can kill tumor cells and secrete cytokines that activate/recruit other immune cells to participate in the antitumor response. APC, antigen-presenting cell; IFN-γ, interferon-γ; MHC, major histocompatibility complex; PD-1, programmed death-1; PD-L1, PD ligand 1; TCR, T-cell receptor. Reprinted from ref. 39.

Table 1 Characteristics of PD-1 checkpoint inhibitors

Compound name	Description of molecule	Mechanism of action	Company
AMP-224	Recombinant fusion protein: extracellular domain of PD-L2 and the Fc region of human IgG	Binds to PD-1; depletion of PD-1 high- expressing T cells (exhausted effector cells)	Amplimmune/ GlaxoSmithKline
BMS-936559	High-affinity, fully human, PD-L1-specific, IgG4 monoclonal antibody	Blocks binding of PD-L1 to PD-1 and CD80	Bristol-Myers Squibb
MEDI4736	Fully human, high-affinity monoclonal anti-PD-L1 antibody	Blocks binding of PD-L1 to PD-1 and CD80	MedImmune
MPDL3280A	Human anti-PD-L1 monoclonal antibody containing an engineered IgG Fc domain to prevent ADCC	Blocks binding of PD-L1 to PD-1 and CD80	Roche/Genentech
Nivolumab	Fully human monoclonal IgG4 antibody against PD-1	Blocks binding of PD-1 to PD-L1 and PD-L2	Bristol-Myers Squibb
Pembrolizumab (MK-3475)	Humanized monoclonal IgG4 antibody against PD-1 variable region sequences of a very-high-affinity mouse antihuman PD-1 antibody grafted into a human IgG4 immunoglobulin, with an alteration to increase stability	Blocks binding of PD-1 to PD-L1 and PD-L2	Merck
Pidilizumab (CT-011)	Humanized anti-PD-1 IgG1 monoclonal antibody	Blocks binding of PD-1 to PD-L1 and PD-L2	CureTech/Teva

ADCC, antibody-dependent cell-mediated cytotoxicity; IgG, immunoglobulin G; PD-1, programmed death-1; PD-L1, PD ligand 1.
From refs. 41–49.

17% (22/129), not including 6 patients with immune-related responses.[50] Half of responding patients showed a response by the first assessment at 8 weeks. The estimated median response duration was 74.0 weeks (range: >6.1 to >133.9 weeks), and responses were ongoing in 45% of patients at the time of analysis. Overall survival was 42% at 1 year and 24% at 2 years.

Nivolumab has nine active clinical trials in NSCLC at the time of writing, including trials in patients with advanced or metastatic solid tumors that include NSCLC.[40] Phase I and I/II trials combine nivolumab with various other therapies including chemotherapies, targeted agents (bevacizumab or erlotinib), or other immunotherapies: IL-21, ipilimumab, anti–lymphocyte activation gene 3, or lirilumab, which targets a key inhibitory receptor on NK cells (killer cell immunoglobulin-like receptor). Furthermore, there are two phase II and two phase III trials of nivolumab in patients with NSCLC only. The phase II studies are

Table 2 Data to date of PD-L1 agents in lung cancer

Compound name	Type	Setting	Phase	Dosing/description	Primary end point(s)	Safety data	Efficacy data	Expected completion date	NCT number, reference
BMS-936559	Anti-PD-L1	Advanced or recurrent solid tumors, including NSCLC	I	Dose escalation, 0.3–10 mg/kg every 2 weeks in 6-week cycles for up to 16 cycles until complete response, progression, unacceptable toxicity, or withdrawal of consent	Safety, maximum tolerated dose, and dose-limiting toxicities	Safety not broken down by tumor type. 207 total patients, including 75 with NSCLC Treatment-related AEs: 61% Grade 3/4 related AEs: 9% Potential irAEs: 39%, including rash, hypothyroidism, and hepatitis	49 Evaluable NSCLC patients ORR: 5/49 (10%); duration >2.3 to >16.6 months Stable disease ≥24 weeks: 6/49 patients PFS at 24 weeks: 31%	December 2013	NCT00729664, ref. 42
MPDL3280A	Anti-PD-L1	Locally advanced or metastatic solid tumors, including NSCLC	I	Dose escalation, 0.03–20 mg/kg every 3 weeks for up to 1 year	Dose-limiting toxicities	85 Evaluable NSCLC patients; treatment-related grade 3/4 AEs: 11%, including fatigue (2%), nausea (1%), dyspnea (1%), and vomiting (1%) 1 case of grade 3 hyperglycemia; no grade 3–5 pneumonitis	53 Evaluable NSCLC patients ORR: 12/53 (23%); duration: >24 to >75 weeks	November 2016	NCT01375842, ref. 45

AE, adverse event; irAE, immune-related adverse event; NSCLC, non–small cell lung cancer; ORR, objective response rate; PD-L1, programmed death ligand 1; PFS, progression-free survival.

From refs. 43 and 46.

investigating (i) nivolumab monotherapy as a third-line treatment in patients with squamous NSCLC (NCT01721759) or (ii) nivolumab therapy following azacitidine and entinostat vs. oral azacitidine in patients with advanced NSCLC (NCT01928576). The phase III trials, both fully enrolled, are comparing nivolumab to docetaxel in previously treated NSCLC; one study involves patients with squamous cell NSCLC (NCT01642004), whereas the other is focusing on patients with nonsquamous histologies (NCT01673867). In addition, a planned phase III trial evaluating nivolumab vs. chemotherapy (investigator's choice) as first-line treatment of PD-L1+ advanced NSCLC will start enrolling patients later this year (NCT02041533), as will a planned phase III nivolumab safety study in patients with advanced NSCLC (NCT02066636).[40]

There is initial evidence that combination strategies that involve immune checkpoint blockade may also have additive effects in the clinic. In patients with advanced melanoma, combination therapy with nivolumab and ipilimumab showed preliminary activity much greater than that seen in previous experience with either agent alone: 40% of patients on a concurrent regimen had an objective response, and 65% had evidence of clinical activity.[52] Results of the ongoing trials of checkpoint inhibitors in lung cancer will provide further insight into new therapeutic targets and inform approaches for checkpoint inhibitor use in patients who currently have limited treatment options.

PRACTICAL IMPLICATIONS OF AN IMMUNOTHERAPEUTIC MECHANISM OF ACTION
Drug disposition and considerations for different patient populations
The approved or in-development immune checkpoint inhibitors are all therapeutic monoclonal antibodies, with the exception of AMP-224, and all are administered as an i.v. infusion (**Table 1**). Because immune checkpoint inhibitors act to stimulate the immune system, patients with a history of autoimmune disease or other diseases requiring immunosuppressive therapy, including corticosteroids at supraphysiological doses, are probably not appropriate candidates for immunotherapy. Such patients were and are excluded from clinical trials.[40,42,44,49,52]

Because the PD-1 pathway inhibitors have, to date, reported phase I data only, limited evidence is available to inform their potential in individual subpopulations of patients. All IgG

Table 3 Data to date of PD-1 agents in lung cancer

Compound name	Type	Setting	Phase	Dosing/description	Primary end point(s)	Safety data	Efficacy data	Expected completion date	NCT number, reference
Pembroli-zumab (MK-3475)	Anti-PD-1	NSCLC patients previously treated with two systemic regimens	I	10 mg/kg every 3 weeks	Safety, response rate, and biomarkers	38 Patients enrolled Drug-related AEs: 53%, including rash (21%), pruritus (18%), fatigue (16%), and diarrhea (13%) One case each of grade 2 hyperthyroidism, hypothyroidism, and pneumonitis; one case of grade 3 pulmonary edema	ORR: 7/33 (21%) Median OS: 51 weeks Median PFS: 9.7 weeks	February 2016	NCT01295827, ref. 43
Nivolumab	Anti-PD-1	Advanced or recurrent malignancies, including NSCLC (pretreated)	I	1–10 mg/kg every 2 weeks for a maximum of 12 cycles (4 doses per 8-week cycle)	Safety and tolerability	129 Evaluable NSCLC patients; treatment-related select AEs: 41%, including skin (16%), gastrointestinal (12%), and pulmonary (7%); treatment-related grade 3/4 select AEs: 5%; drug-related pneumonitis (any grade): 6% (8/129); grade 3/4 pneumonitis: 2% (3/129); 2 deaths from pneumonitis	ORR: 22/129 (17%) Median OS: 9.9 months all NSCLC; 9.2 months squamous NSCLC 10.1 months nonsquamous NSCLC Alive at 1 year: 42% ($n = 16$) squamous; 43% nonsquamous ($n = 26$)	June 2015	NCT00730639, ref. 50
Nivolumab	Anti-PD-1	Stage IIIb/IV NSCLC	I	Monotherapy, maintenance therapy, or in combination with various agents Arm A: nivolumab (10 mg/kg) + gemcitabine + cisplatin, $n = 12$ squamous Arm B: nivolumab (10 mg/kg) + pemetrexed + cisplatin, $n = 15$ nonsquamous Arm C: nivolumab (10 mg/kg) + carboplatin + paclitaxel, $n = 15$ (3 squamous, 12 nonsquamous) Arm C5: nivolumab (5 mg/kg) + carboplatin + paclitaxel, $n = 14$ (1 squamous, 13 nonsquamous); nivolumab was given every 3 weeks until progression. Platinum doublet was given for four cycles at standard dosing	Safety and tolerability	No dose-limiting toxicities seen Treatment-related AEs: A, 25%; B, 47%; C, 73%, C5: 29%; grade 3/4 treatment-related AEs: 45% overall, including pneumonitis (7%), fatigue (5%), acute renal failure (5%), and anemia (4%); four patients had grade 3/4 pneumonitis	ORR: A, 4/12 (33%) B, 7/15 (47%) C, 7/15 (47%); and C5, 7/14 (50%); PFS at 24 weeks: A, 36%; B, 71%; C, 38%; and D, 55%	August 2015	NCT01454102, ref. 51

AE, adverse event; NSCLC, non–small cell lung cancer; ORR, objective response rate; OS, overall survival; PD-1, programmed death-1; PFS, progression-free survival.

From refs. 40,41,44.

antibody subtypes can cross the placenta; therefore, the use of the checkpoint inhibitors will probably be contraindicated in pregnancy.

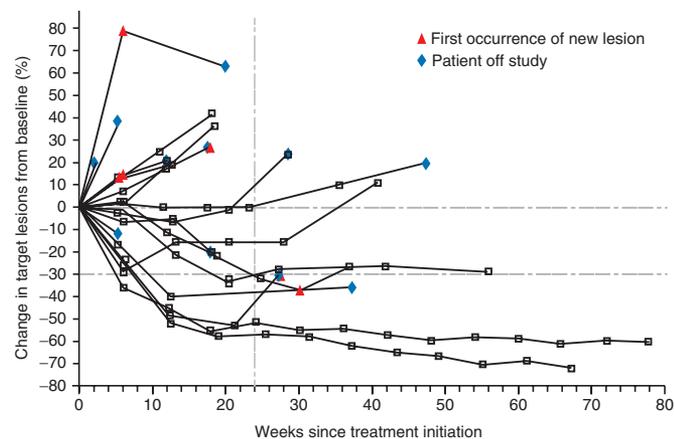## Potential effects on cytochrome P450 enzymes

Drug–drug interactions or toxicity profiles of PD-1 pathway inhibitors in combination with other drugs are not well established. Therapeutic monoclonal antibodies are not metabolized

Figure 2 Activity of anti-PD-L1 antibody in patients with advanced NSCLC. Shown is the tumor burden (assessed as the longest linear dimension) over time in patients with NSCLC who received 10 mg of anti–PD-L1 antibody per kilogram body weight. In most patients who had an objective response, responses were durable and were evident by the end of cycle 2 (12 weeks) of treatment, regardless of the drug dose or tumor type. The vertical dashed line marks the 24-week time point at which the rate of progression-free survival was calculated. Tumor regression followed both conventional and immune-related patterns of response, such as a prolonged reduction in the tumor burden in the presence of new lesions. NSCLC, non–small cell lung cancer; PD-L1, programmed death ligand 1. Reprinted from ref. 42.

by cytochrome P450 (CYP) enzymes but are instead cleared by renal filtration or via receptor-mediated mechanisms.[53] As such, they are not expected to have direct drug–drug interactions involving CYP enzymes.[53,54] However, there is evidence that cytokines involved in effector T-cell responses can alter regulation of many drug transporters and levels of CYP enzymes. Administration of high-dose IL-2 to patients with hepatocellular carcinoma has been shown to decrease expression of multiple CYP enzymes, and administration of IL-2 to cancer patients has been proposed to cause clinically important drug interactions.[53,55,56] Immune-modulating antibodies used in cancer may have cytokine-mediated effects on CYP enzymes, although direct evidence is currently unavailable.

Clinical responses with immunotherapy

The anticancer mechanism of action is different with checkpoint inhibitors as compared with chemotherapy. Standard lung cancer chemotherapies and targeted agents act directly on cancer cells to inhibit tumor growth or cause tumor cell death via apoptosis, necrosis, or both.[57] Chemotherapies work by interrupting DNA synthesis, replication, and/or repair or by inhibiting normal cell division by other means. Targeted agents work to inactivate mutated or overexpressed proteins that confer growth/survival advantages, thereby reducing the aggressiveness of the tumor.[3,58,59] The effectiveness of these standard therapies is measured radiologically by tumor shrinkage.[60]

In contrast to conventional cytotoxic agents, immunotherapies are designed to stimulate antitumor immune responses so that tumors are destroyed via normal immune processes. This antitumor activity occurs by contact-dependent and contact-independent interactions between T cells and tumor cells that are facilitated by a host of other immune cells, including dendritic cells, NK cells, and B cells.[61] As a clinical consequence,

Table 4 Management strategies for irAEs with ipilimumab

Organ system	Strategy
Skin	
Eruptions and pruritus	Symptomatic management with topical creams and oral antipruritics
Grade 2 (persistent)	Withhold a dose
Grade 3	Withhold a dose and treat with a tapering course of oral steroids
Grade 4	Permanently discontinue treatment; initiate steroids for ≥30 day taper
Diarrhea/colitis	
Grade 2[a]	Treat with oral diphenoxylate hydrochloride, atropine sulfate, and budesonide; oral systemically absorbed steroids may be required
Grade 3 or 4	Discontinue treatment, administer i.v. steroids, and replenish fluids and electrolytes; thereafter, oral steroid taper for ≥ 4 weeks. Infliximab followed by long (≥7 week) steroid taper may be required
Hepatotoxicity	
Grade 3–4	Permanently discontinue treatment. Give high-dose i.v. steroids for 1–2 days, followed by oral dexamethasone tapered over ≥30 days
Endocrinopathy	
Grade 3–4	Discontinue treatment. Administer i.v. steroids followed by oral steroid taper for ≥ 4 weeks and replacement of appropriate hormones

irAE, immune-related adverse event.

[a]After infection and inflammatory bowel disease were ruled out.

Adapted from ref. 65.

antitumor responses that are delayed, as compared with those for chemotherapy or targeted agents, may occur because it may take time for an antitumor immune response to be mobilized and prove effective at killing tumor cells.

Immune checkpoint inhibitor trials have also reported pseudoprogression, whereby the tumor volume increases after initiation of checkpoint inhibitor therapy but is then followed by tumor regression or a prolonged reduction in tumor burden in the presence of new lesions (Figure 2).[42,43,45,49,50,62,63] This may be explained by an influx of effector T cells, dendritic cells, and other immune cells into the tumor after activation by immune-modulating therapy, and not by an increase in tumor cells. These unconventional responses may be associated with favorable survival.[62,63]

To better characterize responses with immunotherapy, immune-related response criteria have been defined. With these criteria, in contrast with RECIST criteria, new lesions do not necessarily represent progressive disease. Moreover, instead of using the sum of the product of perpendicular diameters of index lesions to determine tumor burden, the immune-related response criteria calculate an overall tumor burden based on the sum of the product of perpendicular diameters of index and new measurable lesions.[63] Because these immune-related response criteria are still undergoing prospective validation in clinical trials, their usefulness across the spectrum of solid tumors, or even across the spectrum of immune checkpoint inhibitors under development, is an open question. Nevertheless, clinicians and researchers should be aware of the possibility for differences in responses and know how to accurately confirm responses when using immunotherapies so as to make well-informed treatment and investigation decisions.

Response rates with PD-1 pathway inhibitors in NSCLC are evolving; however, it is clear that a substantial proportion of patients achieve clinically meaningful and prolonged responses. Nivolumab monotherapy showed a median response duration of 74.0 weeks (range: >6.1 to >133.9 weeks), and responses were ongoing in 45% of responding patients (10/22) at the time of analysis.[50] In an interim analysis, 7 of the 38 NSCLC patients receiving pembrolizumab (MK-3475) had ongoing responses at >60 weeks.[43] Preliminary data for MPDL3280A showed that responses in 9 of 53 NSCLC patients were ongoing at the time of analysis, with the duration of responses ranging from >24 to >75 weeks.[45] These highly encouraging response data in a population with a historically dismal prognosis support efforts to fully understand and characterize mechanisms of clinical and biological response. Ongoing efforts to refine patient selection are encouraging, and collaborations between pharmacologists and immunologists are critical to elucidating T-cell activation drivers to optimize responses in a greater number of patients.

Monitoring, management, implications, and possible predictors of irAEs

Health-care providers and patients must also be on the alert for irAEs with immune checkpoint inhibitors, which can occur at any time after the start of treatment and which require prompt diagnosis and interventions for effective management.[64,65] Types of irAEs reported with immune checkpoint inhibitors include dermatologic (rash, pruritus, and vitiligo), gastrointestinal (diarrhea and colitis), endocrine (hypothyroidism and hyperthyroidism), and hepatic (hepatitis and increased liver function enzymes) events, as well as pneumonitis, uveitis, infusion-related events, and fatigue.[5,40,42,44,49,52,66]

Across the different immune checkpoint inhibitors, some differences in incidence and type of grade 3/4 irAEs have been observed.[5,42,44,49] Further studies will be needed to determine differences between these therapies. However, trials of the newer checkpoint inhibitors have successfully used approaches to managing irAEs similar to those used for managing ipilimumab-related irAEs (Table 4),[65] which suggests that development of class-wide management principles is reasonable.[42,44,49] General strategies included the administration of corticosteroids for irAEs, a delay in a scheduled dose for moderate irAEs, and discontinuation of therapy for severe reactions. Dose reductions are not recommended for the management of irAEs. Appropriate clinical management is critical for successful outcomes. This includes ruling out perforation in colitis, seeking appropriate consultation (e.g., endocrinology for hypophysitis or adrenal insufficiency or pulmonology for pneumonitis), and increasing the monitoring frequency of patients experiencing irAEs, including monitoring of liver function and timely follow-up of patients with diarrhea or worsening pulmonary symptoms. To date, no clear relationship between the incidence or severity of irAEs and response has been noted, making additional biomarker development necessary for prediction of initial and subsequent response to immune checkpoint therapy.

The effect of dose on the incidence and severity of irAEs is also unclear. In the nivolumab phase I study, no maximum tolerated dose was defined across the 0.1, 1, 3, or 10 mg/kg doses tested.[49] However, patients receiving doses associated with a greater likelihood of response appear to be more likely to discontinue therapy due to an AE throughout treatment.[44,49] Similarly, in the BMS-936559 anti-PD-L1 antibody study, no maximum tolerated dose was defined across the 0.3, 1, 3, or 10 mg/kg doses tested, and the percentages of patients with any grade AE or grade 3/4 AEs were similar across the 1–10 mg/kg doses, with numerically higher rates of all AEs in the 1 mg/kg group.[42]

More challenging is the current lack of predictors of irAEs in a given patient receiving a specific dose. The pharmacokinetic profiles of these IgG monoclonal antibodies show dose linearity. For example, pharmacokinetic results for BMS-936559 reported geometric mean area under the curve (0–14 days) values of 2,210, 7,750, and 36,620 µg/ml/h for doses of 1, 3, and 10 mg/kg, respectively.[42] The coefficient of variation ranged from 34 to 59%. After the first dose, geometric mean peak levels at these dose levels were 27, 83, and 272 µg/ml, respectively (coefficient of variation: 30–34%).

Peripheral pharmacodynamic markers are unlikely to be of benefit because peripheral blood mononuclear cells from 65 patients receiving nivolumab (0.1–10 mg/kg every 2 weeks) showed a median PD-1 receptor occupancy of 64–70% across dose levels.[49] However, relationships between receptor occupancy in peripheral blood and that in other tissues remain

poorly understood, and therefore heterogeneity of occupancy in tumor and target irAE tissues (e.g., colon and liver) may contribute to AEs.

FUTURE DIRECTIONS AND CONCLUSIONS

The use of immunotherapy to unlock the immune system's ability to eradicate cancer cells is an exciting new avenue for treatment of solid tumors, even in an extremely aggressive disease with poor prognosis such as advanced lung cancer, but the exact clinical applications are still not clear. Ongoing trials investigating combination immunotherapy with additional therapeutic agents, the impact of treatment sequence of immunotherapy with chemotherapy, and the possible role of maintenance therapy with checkpoint inhibitors should advance our knowledge and, hopefully, treatment options.

In lung cancer therapy, the emergence of targeted agents with mechanisms of action based on driver mutations have introduced a set of standard predictive biomarkers to aid in clinical decision making. Immune checkpoint blockade, however, is designed to act on a complex and intact immunological pathway rather than individual mutations or antigens; as such, identification of a predictive biomarker has proven challenging. Answers as to whether biomarkers such as PD-L1 can predict tumor responsiveness to agents targeting this pathway have been equivocal so far.

In summary, the goal of immunotherapy is to prolong survival and quality of life for patients with lung cancer by stimulating the patient's own immune system to combat cancer. This new treatment approach, however, is still early in lung cancer, and more data from mechanistic and clinical studies are needed on strategies to optimize the clinical impact of these therapies.

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